# Summary Report on the Effects of EXP60707A (Acetamiprid 20%SP) on Honey Bees (*Apis mellifera* L.)

**PMRA Submission Number N/A** **EPA MRID Number 459325-05**

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**Data Requirement:**

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|---|---|
| PMRA Data Code: | N/A |
| EPA DP Barcode: | Not Provided |
| OECD Data Point: | None |
| EPA Guideline: | None |

**Test Material:** EXP60707 A
Common name: Acetamiprid
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 135410-20-7
Synonyms: Acetamiprid 20%SP

**Purity:** 203 g/kg

**Primary Reviewer:** John Marton
**Staff Scientist, Cambridge Environmental inc.**

**Signature:**
**Date:** 11/05/07

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**{EPA/OECD/PMRA}**

**Date:** 8/19/2008

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**Reference/Submission No.:** {.....}

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|---|---|---|
| **Company Code** | {.....} | [For PMRA] |
| **Active Code** | {.....} | [For PMRA] |
| **Use Site Category:** | {.....} | [For PMRA] |
| **EPA PC Code** | 099050 | |

**Date Evaluation Completed:** {dd-mm-yyyy}

**Citation:** Schur, Andrea. 2002. A Semi-Field Study on the Effects of a Foliar Application of EXP60707 A (Acetamiprid 20%SP) on the Brood Development of the Honey Bee (*Apis mellifera* L.). Unpublished study performed by Arbeitsgemeinschaft, GAB Biotechnologie GmbH, Niefern-Oschelbronn, Germany. Laboratory report number 20011073/01-BZEU. Study sponsored by Nippon Soda Co., Ltd., Chiyoda-ku, Tokyo, Japan. Study completed January 9, 2003.

**SUMMARY:**
The semi-field study was carried out following the EPPO Guideline No. 170: Guideline on methods for evaluating the side-effects of plant protection products on honey bees (EPPO, 1992). The test substance was applied to plots approximately 50 $m^2$ of flowering *Phacelia* at a test rate equivalent to 100 g ai/ha in 400 L water/ha. Plots of the same size were either treated with tap water (control) or with 600 g Insegar 25WG/ha (fenoxycarb) as a toxic standard (effects on brood development). One small bee colony, containing 12 combs with about 6000 honey bees, was placed into each tent. The test was carried out in one run with 3 replicates per treatment.

Mortality, behavior, foraging activity, condition of the colonies and development of the bee brood was assessed before and after treatment. The purpose of this test was to determine if foliar applications of the test material have adverse effects on the pre-imaginal stages of the honey bee (*Apis mellifera* L.). No

adverse effects were detected in this study. However, brood development in the control and treatment groups did not differ appreciably from brood development in the 'toxic standard' treatment.

This study is classified 'supplemental', as it contains information useful for risk assessment purposes, but is not conducted under any Agency guideline.

## METHODOLOGY:

Test Materials:
The test material was the insecticide EXP60707A (formulation containing the active ingredient acetamiprid, 203g/kg) which was described as a light blue powder stored in the dark at a temperature of 2 to 30°C. The toxic standard was the insecticide Insegar 25 WG (formulation containing the active ingredient fenoxycarb, 25.7%) which was described as grey to brown granules stored under ambient conditions.

Test Organism:
The honey bee (*Apis mellifera* L; Hymenoptera, Apidae) was used as the test organism. The honey bee is an important beneficial insect due to it pollination activity in fruit, berry and seed growing. Due to the specific use of honey bees in the crops pollinated (migratory beekeeping) they are an important productive factor. Additionally, they contribute to the preservation of a multitude of wild flowering plants because of their pollination activity.

Test Site:
The field where the study was conducted was located in the south of Germany near Pforzheim in an arable field. The crop used was *Phacelia tanacetifolia* which is recommended in guideline EPPO 170 for tunnel testing. Prior to flowering, 11 areas of approximately 50 $m^2$ of *Phacelia* plants were covered with tunnel frames with light plastic gauze. Each tunnel constituted an experimental plot. The size of each tunnel (covered plot) was 12 m long, 5 m wide and 3.5 m high in the center. A water supply was placed into each tent for the bees and traps were installed in front of the hives to determine the number of dead bees at the entrance.

Experimental Bee Colonies:
Small healthy colonies ("Mini-Plus-Beuten"; 1 queen and approximately 6000 bees per colony) with 12 combs were used for the test. All nuclei were produced at the same time. Additionally, the following criteria for each nuclei were met:

- at least six brood combs containing all brood stages were present
- at least one honey and pollen comb were present
- bees were free of symptoms of *Nosema* and other bee diseases

Each hive contained 2 bodies with one bottom and one lid (height: 40 mm). The outer dimensions were 300 x 300 mm and the inner dimensions were 230 x 230 mm and the height was 170 mm. Each body contained 6 frames (130 x 200 mm).

To register dead bees which were carried out of the hives, wooden bee traps with gauze on the bottom and top were attached to the entrance. The hives were placed into the tents 6 days before the planned application of the test material to allow the bees to familiarize with the environment and to lower the mortality, which is usually increased due to stress from transport. The applications were carried out once mortality stabilized around normal levels. An open container containing at least 4 L of water was placed into each tent. Scraps of polystyrene covered the surface of the water to prevent the bees from drowning. Furthermore, mortality within the crop was recorded. To do this, plants were removed along the edges of each plot of the same width (0.6 m) all around and water permeable linen sheets (area approximately 20 $m^2$) were spread out on the surface to count the number of dead bees in the plots.

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Experimental Design and Layout:
A total of 11 tents were set up in the field with three tents each for the control, test material treatment group and toxic standard treatment group. An additional tent was set up for the control and test material treatment group. The two additional tents were used for collection of pollen samples; no biological assessments were made in these two tents. In the tents where biological assessments were made, pollen collection did not occur to avoid any potential adverse effects that the methodology for pollen collection might have on the normal provision and storage of pollen in the interior of the hive. Pollen samples were collected by placing a pollen trap at the entrance of each hive.

Seven days after application of the test material, the bee colonies were taken out of the tents and the hives were transferred to an area without main crops which are attractive for bees and where no pesticides were used the time of assessments.

Application:
Test solutions were prepared shortly before each application. The applications were carried out up to midday with a plot sprayer that emulates a commercial application. During all applications, the *Phacelia* crop was in full bloom, bees were actively foraging and the wind speed was below 2 m/sec. The sprayer was calibrated before the application.

Assessments:
Mortality was assessed by observation of the wooden bee traps to determine the number of dead bees carried out of the hive and by observation of the linen sheets spread around the *Phacelia* plants. Mortality was assessed once a day beginning five days prior to application, three times on the day of application, three times on the day following application and once daily for seven days after application. Mortality of the adult honey bees in the bee trap at the entrance of the colonies was assessed for an additional 13 days after transferring the colonies from the tents (until 22 days after the brood area fixing day; BFD).

Flight intensity was determined by counting the number of bees that were both foraging on flowering crops and flying immediately over the crop within a 1 $m^2$ section during a one-minute observation period. The square observed was chosen at random. Assessments were made once a day beginning five days prior to application, immediately before application, 1, 2, 4 and 6 hours after application, and daily for seven days after application.

The assessments of condition of the colonies and the brood development were done on the same date and time to avoid negative effects by opening the hives more often than necessary. The condition of the colonies was checked once before the application and five times after the application. The condition and development of the bee brood were assessed using the following parameters:

- strength of colony (number of combs covered with bees)
- presence of a healthy queen (presence of eggs, presence of queen cells)
- estimate of the pollen storage area and area with nectar (in % of the comb area)
- estimate of the area containing eggs, larvae and capped cells (in % of the comb area)

At each assessment both sides (together) of one comb were assigned to be 100% and the percentage area containing the brood stages, pollen and nectar on the comb was estimated. This was done for all combs per hive. Afterwards, the mean values were calculated for each hive and assessment date.

The assessment of development of the bee brood in individual marked brood cells was carried out by using acetate sheets. At each assessment before the application (=BFD) a brood comb was taken out of each colony to mark areas with at least 100 cells containing eggs. An attempt was made to mark up to 150 cells with eggs per hive. The exact situation of each cell and its content was marked in the acetate sheet. The sheet was fixed with needles on the wooden frame and the position on the frame was marked. This allowed placing the sheet exactly in the same position on each of the following observation dates. Therefore, the

development of each individually marked cell throughout the duration of the study was determined (pre-imaginal development period of worker honey bees typically averages 21 days).

Observations were made for the following brood stages: eggs, young larvae (L1-L2), old larvae (L3-L5), pupae (capped cell), nectar, pollen, dead larvae/pupae, or empty. A numerical value was assigned to each development stage: 0 (empty at the beginning/termination of the development), 1 (egg stage), 2 (larval stage), 3 (colled larval), 4 (pupal stage) and 5 (empty after hatch). To compare the different treatment groups, a brood index was calculated using the values assigned to the single cells in each treatment group. All values from each treatment group, assessed at the same date, were summed up and divided by the number of observed cells in order to obtain the brood index.

The behavior of the bees on the crop during exposure inside the tents and around the hive was observed prior to application and after application once a day.

Samples of pollen were taken from pellets collected by worker bees in front of the hives and directly from the *Phacelia* flowers. The pollen gathered by the worker bees while foraging inside the tents was collected using a pollen trap set in front of one hive. The pollen trap was operational for at least 5 hours per day on each of the sampling days. The amount of anthers with pollen collected directly from the flower in each tent and sampling data was approximately 1 gram. A total of 5 pollen samples were collected. The first was taken three days prior to application of the test material; the second was taken on the day of application; the third was taken 5 days after application; the fourth was taken on the last day hives were inside the tunnels; and the fifth sample was taken directly from the flowers at the end of the flowering period 7 days after the fourth sample.

Evaluation of Results:
The influence of the test substance was evaluated by comparing the bees in the treatment tents to the control bees and toxic standard treatment group with the following observations:

- Mortality at the edge of the treated area and in the bee traps
- Foraging activity (number of forager bees/minute/$m^2$ flowering *Phacelia* crop)
- Behavior of the bees on the crop and around the hive)
- Development of the bee brood

**RESULTS:**

Mortality:
For the five days prior to application mean mortality (bee trap + linen sheets along edge of treated area) from all tents averaged 18.6 bees/day/tent in the control, 22.8 bees/day/tent in the treatment group and 15.5 bees/day/tent in the toxic standard treatment. Twenty-two days after application, mean mortality was 13.0 bees/day/tent in the control, 13.4 bees/day/tent in the treatment group, and 19.4 bees/day/tent in the toxic standard treatment.

The study author's analysis of the mortality data did not detect any significant differences in the number of dead bees at the single treatment level on the linen sheet at any point during the definitive test, relative to the negative control. Significant differences in mortality at the treatment level were detected in the bee trap five days prior to the application of the test material, on the day of application, two days after application and 20 days after application. No other significant differences in mortality were detected.

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**Table 1. Mortality of *Apis mellifera* before and after exposure of EXP60707A**

| Treatment Group | Pre-Application | | | Post-Application | | |
|---|---|---|---|---|---|---|
| | Mean Bee Trap | Mean Linen | Mean/Tent and Day | Mean Bee Trap | Mean Linen | Mean/Tent and Day |
| Control | 2.5 | 16.1 | 18.6 | 4.5 | 22.3 | 13.0 |
| Treatment Group | 4.9 | 17.9 | 22.8 | 4.8 | 22.5 | 13.4 |
| Toxic Standard | 3.3 | 12.2 | 15.5 | 12.8 | 17.4 | 19.4 |

The mean bee trap and mean linen values represent the mean of dead bees observed each day between the two tents. The Mean/Tent and Day value is the overall mean value of dead bees observed in the bee trap and on the linen from both tents over the entire duration.

Flight Intensity:
Five days prior to application, flight intensity between the three tents averaged 7.4 bees/minute/$m^2$ in the control group, 9.7 bees/minute/$m^2$ in the treatment group, and 9.4 bees/minute/$m^2$ in the toxic standard group. On the day of application, flight intensity changed to 10.1, 10.2 and 14.8 bees/minute/$m^2$ in the control, treatment group and toxic standard group, respectively. One day after application the flight intensity was 11.1, 12.8 and 11.1 bees/minute/$m^2$ in the control, treatment group, and toxic standard group, respectively. By test termination, flight intensity was 7.2, 8.0 and 7.9 bees/minute/$m^2$ in the control, treatment group, and toxic standard group, respectively.

No behavioral differences in front of the hives or in the crop of the tents were observed in the test substance treatment compared to the control treatment.

**Table 2. Flight intensity of *Apis mellifera* before and after exposure of EXP60707A**

| Treatment Group | Pre-Application (bees/minute/$m^2$) | | | | Post-Application (bees/minute/$m^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Tent 1 | Tent 2 | Tent 3 | Mean | Tent 1 | Tent 2 | Tent 3 | Mean |
| Control | 6.7 | 6.8 | 8.6 | 7.4 | 6.5 | 7.5 | 7.6 | 7.2 |
| Treatment Group | 8.9 | 10.9 | 9.2 | 9.7 | 8.2 | 7.7 | 8.2 | 8.0 |
| Toxic Standard | 9.4 | 9.1 | 9.5 | 9.4 | 8.0 | 8.5 | 7.3 | 7.9 |

Brood Development:
At the first brood assessment, the number of combs covered with bees (strength) was between 8.5 and 10.0 in the colonies of the treatments. Observations made after applications showed that the strength only slightly changed. No test substance-related difference in the strength of the colonies could be observed in the test substance treatment compared to the control treatment. From 8 to 20 days after application, strength of two colonies in the toxic standard group slightly decreased to 8.00. In colony 1 of the control group, the loss of the queen resulted in a decline in the number of combs containing brood 20 days after application. The other two colonies in the control remained stable or changed slightly during the test. In all colonies of the toxic standard treatment, a decline in the number of combs covered with brood was observed up to the end of the observation period.

Nearly every colony in the control, treatment, and toxic standard tents exhibited fluctuations in the relative amount of different pre-imaginal stages (egg stage, larval and pupal stage) during the observation period. The loss of the queen in colony 1 of the control results in a lack of eggs 8 days after application. It was possible that the queen was damaged during brood assessment 3 days after application. To produce a new queen, queen cells were built by the worker bees which were noticed 14 days after application. It was determined that a new queen was not produced as no eggs were observed at the following assessments.

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Decreased amount of larvae, eggs and capped cells were observed in different tents of the toxic standard group following application.

Observations of the individually marked cells to determine brood indicated that colonies 1 and 2 from the treatment group and colonies 1 and 2 from the control experienced a normal brood development with rising brood indices from the brood fixing day to 16 days later. This shows that in these groups, most of the eggs which were marked at the start of the test developed to larvae and further to pupae. According to the life-cycle of a worker bee which normally averages 21 days, it can be assumed that young bees hatched between 16 and 22 days after the brood fixing day (14 to 20 days after application of the test material) and the marked brood cells were empty of filled again with eggs or larvae. Based on the percentage of bee brood that failed to complete their full development, it was determined complete successful development was 91 and 86% in two control tents and 81 and 79% in two treatment level tents. The toxic standard (fenoxycarb) impeded the normal development of almost all marked brood cells as the termination rate was 93.7, 100 and 100% in the three tents.

**Table 3. Brood development of *Apis mellifera* before and after exposure of EXP60707A**

| Assessment | Brood Development | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Treatment Group | | | Toxic Standard | | |
| | Tent 1 | Tent 2 | Tent 3 | Tent 1 | Tent 2 | Tent 3 | Tent 1 | Tent 2 | Tent 3 |
| **1st Brood Assessment (prior to application)** | | | | | | | | | |
| Strength[a] | 9.50 | 9.00 | 10.00 | 9.50 | 9.50 | 8.50 | 10.00 | 9.50 | 9.50 |
| # of Combs | 6.00 | 7.00 | 7.00 | 7.00 | 6.00 | 9.00 | 7.00 | 7.00 | 8.00 |
| Egg[b] | 19.17 | 19.29 | 19.29 | 13.57 | 13.33 | 15.56 | 15.71 | 14.29 | 16.25 |
| Larval[c] | 15.83 | 17.86 | 19.29 | 14.29 | 20.83 | 23.89 | 22.86 | 21.43 | 20.00 |
| Capped[d] | 30.00 | 34.29 | 33.57 | 35.71 | 29.17 | 21.67 | 50.00 | 30.71 | 30.63 |
| **2nd Brood Assessment (3 Days After Application)** | | | | | | | | | |
| Strength[a] | 9.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| # of Combs | 6.00 | 7.00 | 7.00 | 7.00 | 6.00 | 8.00 | 7.00 | 7.00 | 8.00 |
| Egg[b] | 15.83 | 16.43 | 10.00 | 13.57 | 12.50 | 13.13 | 13.57 | 11.43 | 9.38 |
| Larval[c] | 27.50 | 13.57 | 19.29 | 22.14 | 19.17 | 15.63 | 9.29 | 7.14 | 3.75 |
| Capped[d] | 36.67 | 40.71 | 39.29 | 37.86 | 47.50 | 38.75 | 52.86 | 42.14 | 41.25 |
| **3rd Brood Assessment (8 Days After Application)** | | | | | | | | | |
| Strength[a] | 10.00 | 10.00 | 10.00 | 8.00 | 9.00 | 9.00 | 9.00 | 8.00 | 8.00 |
| # of Combs | 6.00 | 7.00 | 7.00 | 7.00 | 6.00 | 8.00 | 7.00 | 6.00 | 8.00 |
| Egg[b] | 0.00 | 18.57 | 17.14 | 10.00 | 10.00 | 8.13 | 18.57 | 9.17 | 5.63 |
| Larval[c] | 10.00 | 4.29 | 1.43 | 13.57 | 11.67 | 3.75 | 5.00 | 0.00 | 2.50 |
| Capped[d] | 41.67 | 32.86 | 39.29 | 25.00 | 46.67 | 37.50 | 32.14 | 25.00 | 23.13 |
| **4th Brood Assessment (14 Days After Application)** | | | | | | | | | |
| Strength[a] | 10.00 | 9.00 | 10.00 | 9.00 | 9.00 | 10.00 | 10.00 | 8.00 | 8.00 |
| # of Combs | 6.00 | 7.00 | 7.00 | 7.00 | 6.00 | 7.00 | 6.00 | 6.00 | 6.00 |
| Egg[b] | 0.00 | 26.43 | 22.86 | 11.43 | 18.33 | 22.86 | 12.50 | 15.83 | 17.50 |
| Larval[c] | 0.00 | 26.43 | 24.29 | 22.14 | 16.67 | 17.14 | 17.50 | 14.17 | 17.50 |
| Capped[d] | 40.00 | 24.29 | 24.29 | 37.14 | 31.67 | 27.86 | 16.67 | 5.83 | 14.17 |
| **5th Brood Assessment (20 Days After Application)** | | | | | | | | | |
| Strength[a] | 8.00 | 9.00 | 10.00 | 9.00 | 9.00 | 9.00 | 9.00 | 8.00 | 8.00 |
| # of Combs | 4.00 | 7.00 | 7.00 | 7.00 | 6.00 | 7.00 | 6.00 | 5.00 | 4.00 |
| Egg[b] | 0.00 | 20.00 | 19.29 | 20.00 | 15.83 | 20.71 | 14.17 | 16.00 | 21.25 |
| Larval[c] | 0.00 | 24.29 | 32.14 | 15.00 | 24.17 | 19.29 | 9.17 | 32.00 | 30.00 |
| Capped[d] | 15.00 | 32.14 | 30.71 | 32.14 | 35.00 | 33.57 | 13.33 | 25.00 | 27.50 |
| **6th Brood Assessment (25 Days After Application)** | | | | | | | | | |
| Strength[a] | 9.00 | 9.00 | 9.50 | 9.00 | 8.50 | 9.00 | 10.00 | 8.50 | 7.00 |
| # of Combs | 0.00 | 7.00 | 9.00 | 7.00 | 7.00 | 7.00 | 5.00 | 5.00 | 4.00 |
| Egg[b] | 0.00 | 18.57 | 10.00 | 13.57 | 7.14 | 12.86 | 13.00 | 13.00 | 18.75 |
| Larval[c] | 0.00 | 17.86 | 12.78 | 15.00 | 15.00 | 10.71 | 5.00 | 13.00 | 16.25 |
| Capped[d] | 0.00 | 34.29 | 38.89 | 27.86 | 37.14 | 30.00 | 16.00 | 35.00 | 31.25 |

[a] Strength was determined by the number of combs covered with bees
[b] Average amount (%) of egg stage
[c] Average amount (%) of larval stage
[d] Average amount (%) of capped stage

Pollen Samples:

Residue analyses are not required by guideline OEPP/EPPO No. 170. In this study, samples of pollen were collected in the control and treatment level plots, but the decision on whether to conduct chemical analysis was left pending to the results of the biological part of the study. The rationale was that if the biological observations resulted in conclusive results on the lack of adverse effects of the test material on the development of the brood corroborated by harmful effects on the bee brood by a known insect growth

regulator (fenoxycarb, the toxic standard), there was no need for chemical analysis of the pollen samples. This was the case in this study and therefore, at the decision of the Sponsor, analyses of pollen samples were not carried out.

**REPORTED STATISTICS:**

The statistical software program SAS Version 8 was used for the statistical analysis. The mortality data (dead bee trap and linen sheets) from the test substance and toxic standard treatment were analyzed for significant differences in comparison to the control treatment. This was decided by t-Test (one side higher, probability 95%, $\alpha$=0.05). The homogeneity of data distribution was tested following Shapiro Wilks test.

**CONCLUSION:**

The test material (EXP60707A; formulation containing the active ingredient acetamiprid) resulted in no adverse effects on the mortality of honey bees, or on the flight activity of the bees *Phacelia* plants treated at an application rate of 100 g ai/ha in 400 L water/ha when the bees were actively foraging. No deleterious effects on the overall condition of the colonies during the 25 days following application were observed. Detailed observations of the individual cells for 27 days after the brood fixing day showed that the treatment with the test material did not adversely affect the development of eggs into adult bees. However, the brood development in the control and treatment groups also did not differ appreciably from development in the 'toxic standard'.

**References:**

OEPP/EPPO (2001): Guideline for the efficacy evaluation of plant protection products- Side effects on honeybees. OEPP/EPPO, PP 1/170 (3) update 2000, 19-23.

SAS Institute Inc. (Ed.): SAS/STAT User's Guide Version 8, Fourth Editions. Cary, NC, USA.